Claim 1 has been amended to recite isolation of cells that "express KDR on their surface (KDR^+ cells) from cells that do not express KDR on their surface." Support for this recitation is found in the specification, for example at page 14, lines 11-15.

Multiple recitations of "human," "hematopoietic," "embryonic," and "fetal" have been removed from claims 2-5, 19-22, and 27. Because each of these terms modifies an otherwise uniquely identified element, removal of these terms does not alter the meaning of the recited elements, and is believed to render the claims more readable.

Claims 18, 51, and 69 have been amended to recite preparation of hematopoietic cells by isolating cells which express KDR on their surface. Support for this recitation is found in the specification, for example at page 14, lines 11-15.

Claim 39 was amended to recite that the HPCs are isolated "by removing $CD34^+$ cells," and amended claim 40 was amended to recite that HPCs are isolated "by removing lin^+ cells." Support for the separating $CD34^+$ from $CD34^-$ cells and lin^+ from lin^- cells is found in the specification, for example at page 21, lines 21-22.

Claims 25 and 40 have been amended to recite using an antibody to a "known" lineage marker. Support for this recitation is found in the specification, for example at page 27, lines 27-29. The Applicants also contend that recitation of 'lineage markers' inherently discloses that the markers are known as such.

Claim 51 recites that KDR^+ HSCs are incubated with vascular endothelial growth factor (VEGF), which is a growth factor. Claims 52 and 53 were amended to recite use of another (*i.e.*, in addition to VEGF) growth factor. Use of VEGF in combination with other growth factors is supported in the specification, for example at page 29, line 26.

In claim 53, "receptor " was deleted from the phrase "flt3 receptor ligand" and from the phrase "kit receptor ligand" for clarification. The skilled artisan would understand that flt3 ligand is the ligand of the flt3 receptor, and that kit ligand is the ligand of the kit receptor.

Amended claim 69 recites that an isolated stem cell is "capable of" giving rise to other cells. Support for this recitation is found in the specification, for example at page 30, line 17 through page 31, line 2.

New claims 71-74 depend from claim 69. The recitations in claims 71-74 mirror originally filed claims 2-5 and 19-22.

New claim 75 depends from claim 18 and merely includes subject matter deleted from claim 23.

For the reasons set forth above, the Applicants respectfully contend that the amendments and additions made herein do not include new matter.

Claim Rejections – 35 U.S.C. § 112, first paragraph

Claims 9, 25, 31, 32, 40-44, and 68 are rejected pursuant to 35 U.S.C. § 112, first paragraph.

Claim 68 has been cancelled.

Claims 9, 31, 32, and 44 recite use of the monoclonal antibody (mAb) 260.4. The Examiner contends that the specification does not disclose a repeatable process to obtain the mAb and that it is not apparent if the hybridoma that produces the mAb is readily available to the public. The specification discloses at page 22, lines 1-2 and at page 40, lines 16-18 that mAb 260.4 is available from Gesellschaft für Biologische Forschung (GBF), Braunschweig, Germany. Additionally, this mAb is sold as product number V3003 from Sigma-Aldrich (see the enclosed Exhibit A for product information). Thus, no deposit is believed to be necessary.

In the Examiner's view, the term "lin," as used in claims 25 and 40-44, defines a potentially limitless combination of cell-specific markers. The Applicants note that the terms "lin" and "lin$^-$" refer to lineage markers of cells of the hematopoietic system as disclosed in the specification, for example at page 7, lines 7-9. In the context of the hematopoietic system, the terms "lin" and "lin$^-$" are commonly used and understood in the art, as evidenced by several references of record. For example, each of the Bhatia, Matthews, Sutherland, and Zanjani references uses the term "lin" or "lin$^-$" in reference to lineage markers of hematopoietic cells. Claims 25 and 40 recite known lineage markers. The Applicants respectfully contend that the appropriate lin markers depend on the identity of the cell to be manipulated, and that one skilled in the art would know which lineage markers to use in order to avoid isolating differentiated hematopoietic cells.

For the foregoing reasons, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 9, 25, 31, 32, and 40-44 pursuant to 35 U.S.C. § 112, first paragraph.

Claim Rejections - 35 U.S.C. 112, second paragraph

The Examiner rejects claims 2, 9, 25, 31, 32, and 39-44 pursuant to 35 U.S.C. § 112, second paragraph.

The Examiner objects to the use of the term "pre-embryonic hematopoietic tissue" in claim 2. Amended claim 2 does not recite this term.

In the Examiner's view, recitation of the designation "260.4" for the mAb in claims 9, 31, and 44 is indefinite. As described above, in reference to the rejection of claims 9, 31, and 44 pursuant to 35 U.S.C. § 112, first paragraph, "260.4" is the name of a publicly available mAb. As shown in Exhibit A, this mAb is currently available from Sigma-Aldrich as product number V3003.

The Examiner objects to the use of the terms "lin" (claim 25) and "lin$^-$" (claims 40 and 41). The Applicants respectfully contend that these terms are commonly used in the art as discussed in the section regarding the rejection of claims 25, 40, and 41 pursuant to 35 U.S.C. § 112, first paragraph. commonly used but have no common meaning

In the Examiner's view, there is no antecedent basis for reciting "a population of CD34$^-$ cells" in claim 39. Amended claim 39 does not include this phrase.

For the foregoing reasons, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 2, 9, 25, 31, 32, and 39-44 pursuant to 35 U.S.C. § 112, second paragraph.

Claims Rejections – 35 U.S.C. § 102

Claims 1-5, 18, 19, 21-27, 39, 40, 51-53, and 67 are rejected pursuant to one or both of 35 U.S.C. §§ 102(a) and 102(b) in view of one or more of Bhatia, Sutherland, Zanjani, and Brandt. In the Examiner's view, at least some of the cells disclosed in these references may express KDR.

Claim 67 has been cancelled.

Each of amended claims 1, 18, and 51 recites that cells are selected on the basis that they express KDR on their surface. Amended claims 2-5 depend from amended claim 1, amended claims 19, 21-27, 39, and 40 depend from amended claim 18, and amended claims 52

and 53 depend from amended claim 51. Therefore, each of claims 1-5, 18, 19, 21-27, 39, 40, and 51-53 recite the selection of cells based on expression of KDR on their surface.

Claim 69 is rejected pursuant to 35 U.S.C. § 102(b) in view of Matthews. The Examiner contends that Matthews teaches isolation of stem cells from fetal mouse liver and that Flk-1 (*i.e.*, KDR) is expressed in highly purified stem cells obtained from fetal liver. The Examiner further states that Matthews discloses that stem cells that express Flk-1 are both hematopoietic and non-hematopoietic in nature, and that 14 day old fetuses express Flk-1 in (non-hematopoietic) liver (oval cell) and cardiac (muscle) cells. Amended claim 69 recites isolating cells based on the expression of KDR on their surface.

None of Bhatia, Sutherland, Zanjani, Brandt and Matthews discloses selection of hematopoietic progenitor cells based on expression of KDR on the surface thereof, as recited in claims 1-5, 18, 19, 21-27, 39, 40, 51-53, and 69. Therefore, none of these references teaches every element that is recited in any of these claims. The Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-5, 18, 19, 21-27, 39, 40, 51-53, and 69 pursuant to one or both of 35 U.S.C. §§ 102(a) and 102(b) in view of one or more of Bhatia, Sutherland, Zanjani, Brandt and Matthews references.

Claim Rejections – 35 U.S.C. § 103

Claims 18, 26, 39, and 40 are rejected pursuant to 35 U.S.C. § 103(a) over Osawa and claims 18-20 are rejected pursuant to 35 U.S.C. § 103(a) over Kabrun. The Examiner contends that Osawa teaches isolation of murine long-term repopulating $CD34^-lin^-$ cells, and Kabrun teaches isolation of $Flk\text{-}1^+$ early hematopoietic stem cells from murine embryonic yolk sac. In the Examiner's view, some of the cells isolated according to the teachings in Osawa or Kabrun may be KDR^+. Furthermore, the Examiner states that even though the teachings of Osawa and Kabrun were performed in mouse, it would have been obvious to isolate the same populations of cells from a human subject. The Examiner further asserts that the findings regarding hematopoietic stem cells in mice are applicable to human stem cells. The Applicants respectfully contend that an ordinarily skilled artisan would not have been motivated to perform the methods of either Osawa or Kabrun in a human subject, and that an ordinarily skilled artisan

would not expect success in such methods, for at least two reasons discussed separately in the two ensuing paragraphs.

First, Osawa highlights a difference between the human and murine hematopoietic cells (see the abstract in particular), in that murine HSCs were detected in the CD34 low to negative fraction. As disclosed in the specification, for example at page 55, lines 1-2, human HSCs are $CD34^+$. Furthermore, it is known human $CD34^+$ hematopoietic stem cells are highly quiescent (see Larochelle et al., submitted with IDS) whereas murine $CD34^+$ stem cells are actively cycling (see Sato et al., enclosed with this response). For these reasons, an ordinarily skilled artisan would not extrapolate data obtained using murine hematopoietic cells to predict features of human hematopoietic cells. For the same reasons, an ordinarily skilled artisan would not expect to obtain similar results in human cells.

Second, Kabrun teaches that murine $Flk\text{-}1^+$ cells did not respond to VEGF treatment. In contrast, the treatment of human KDR^+ hematopoietic cells with VEGF results in a significant increase in cell number, as disclosed in the specification, for example at page 24, line 28 to page 25, line 1 and at page 39, lines 19-22. Thus, the cells described in Kabrun either did not comprise KDR or are not a relevant model of human hematopoietic cells. Therefore, an ordinarily skilled artisan would appreciate that it is unreasonable to expect that data regarding murine hematopoietic cells could be used to predict similar findings in human hematopoietic cells.

For the foregoing reasons, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 18-20, 26, 39, and 40 pursuant to 35 U.S.C. § 103(a).

Summary

The Applicants submit that each rejection of the claims has been either overcome or is now inapplicable, and that each of claims 1-11, 18-32, 39-44, 51-53, 69, and 71-75 is now in condition for allowance. Reconsideration and allowance of each of these claims are respectfully requested at the earliest possible date.

Respectfully submitted,

CESARE PESCHLE *ET AL.*

2 July 2001 By: _____
(Date)

GARY D. COLBY, Ph.D., J.D.
Registration No. 40,961
AKIN, GUMP, STRAUSS, HAUER & FELD, L.L.P.
One Commerce Square
2005 Market Street - 22nd Floor
Philadelphia, PA 19103-7086
Telephone: (215) 965-1200
Direct Dial: (215) 965-1285
Facsimile: (215) 965-1210
E-Mail: gcolby@akingump.com

GDC:KML

Enclosures
Petition for Extension of Time
Clean Copy of Substitute Paragraphs, As Amended
Marked-Up Copy of Substitute Paragraphs, As Amended
Clean Copy of Claims, As Amended
Marked-Up Copy of Claims, As Amended
Exhibit A: Sigma Chemical Company Product Information
Sato et al., 1999, Blood 94:2548-2554 (included with enclosed Supplemental Information Disclosure Statement)